FEBS 26588 FEBS Letters 531 (2002) 81–87

Minireview

Dynamics of plasma membrane microdomains and cross-talk to the insulin signalling cascade

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Received 5 May 2002; revised 23 July 2002; accepted 6 September 2002

First published online 18 September 2002

Edited by Edward A. Dennis, Isabel Varela-Nieto and Alicia Alonso

Abstract The critical role of the heterogeneous nature of cellular plasma membranes in transmembrane signal transduction has become increasingly appreciated during the past decade. Areas of relatively disordered, loosely packed phospholipids are disrupted by hydrophobic detergent/carbonate-insoluble glycolipid-enriched raft microdomains (DIGs) of highly ordered (glyco)sphingolipids and cholesterol. DIGs exhibit low buoyant density and are often enriched in glycosylphosphatidylinositol-anchored plasma membrane proteins (GPI proteins), dually acylated signalling proteins, such as non-receptor tyrosine kinases (NRTKs), and caveolin. At least two types of DIGs, hcDIGs and lcDIGs, can be discriminated on basis of higher and lower content, respectively, of these typical DIGs components. In quiescent differentiated cells, GPI proteins and NRTKs are mainly associated with hcDIGs, however, in adipose cells certain insulin-mimetic stimuli trigger redistribution of subsets of GPI proteins and NRTKs from hcDIGs to lcDIGs. Presumably, these stimuli induce displacement of GPI proteins from a GPI receptor located at hcDIGs whereas simultaneously NRTKs dissociate from a complex with caveolin located at hcDIGs, too. NRTKs are thereby activated and, in turn, modulate intracellular signalling pathways, such as stimulation of metabolic insulin signalling in insulin-sensitive cells. The apparent dynamics of DIGs may provide a target mechanism for regulating the activity of lipid-modified signalling proteins by small drug molecules, as exemplified by the sulfonylurea, glimepiride, which lowers blood glucose in an insulin-independent fashion, in part.

Key words: Glycosylphosphatidylinositol-anchored proteins; Lipid rafts; Caveolae; Lipid modification; Non-receptor tyrosine kinases

1. Plasma membrane heterogeneity and microdomains

It is currently being recognized that certain transmembrane signalling functions may be accomplished within compartments present in the plasma membrane as microdomains. Biological membranes exist in distinct phases, microdomains, where the concentration of membrane lipids and/or proteins, as well as their physicochemical properties, are different from the surrounding environment [1–3]. 'Bulk membranes' are enriched in glycerophospholipids (frequently containing unsaturated fatty acids), loosely packed in a liquid-disordered state and therefore display high fluidity. In contrast, microdomains have a high content of glycerophospholipids, (glyco)sphingolipids and glycosylphosphatidylinositol (GPI) lipids (both bearing predominantly saturated fatty acids) as well as cholesterol, tightly packed in a highly-structured liquid-ordered state [4–6]. Cholesterol is thought to contribute to the tight packing of lipids in liquid-ordered microdomains by filling interstitial spaces between other lipid molecules [7,8]. These characteristics form the basis for resistance of microdomains toward solubilization by cold non-ionic detergents or alkaline sodium carbonate as well as their sensitivity toward cholesterol depletion by cholesterol-chelating agents or enzymatic cholesterol degradation as has been observed in most but not all cases studied so far (see below). The insoluble and (often) cholesterol-dependent membrane subfractions/complexes exhibit low buoyant density during sucrose gradient equilibrium centrifugation and are now often referred to as glycolipid-enriched membrane microdomains, detergent-resistant microdomains, membrane lipid rafts or detergent/carbonate-insoluble glycolipid-enriched raft microdomains (DIGs) [2,9]. Their isolation by detergent-free techniques [10–12] proves that they do not originate from artefacts due to the detergent/carbonate-treatment. Rather, DIGs must have been present in the membrane before these treatments and assembled as self-organizing structures [13,14].

2. Microdomains: DIGs and caveolae

DIGs appear to play important roles in intracellular and transmembrane signalling. A steadily increasing number of reports demonstrates the (co)assembly of various signalling proteins at DIGs. Lipid-modified signalling proteins, among them (dually) acylated small and heterotrimeric G proteins and non-receptor tyrosine kinases (NRTKs) as well as GPI-anchored plasma membrane proteins (GPI proteins) cofrac-

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Abbreviations: CBD(P), caveolin-binding domain (peptide); CSD(P), caveolin-scaffolding domain (peptide); DIGs, detergent/carbonate-insoluble glycolipid-enriched raft microdomains; hc/lcDIGs, DIGs of high/low cholesterol and caveolin content; Gce1, GPI-anchored cAMP-binding ectoprotein-1; GPI, glycosylphosphatidylinositol; GPI proteins, GPI-anchored plasma membrane protein; IRS, insulin receptor substrate protein(s); NEM, *N*-ethylmaleimide; NRTK, non-receptor tyrosine kinase; Nuc, 5′-nucleotidase; PIG(-P), phosphoinositolglycan(-peptides)

0014-5793/02/$22.00
PII: S0014-5793(02)03402-6

tionate to a variable extent with (biochemically defined) DIGs from which they can be coimmunoprecipitated with one another [14–16]. For GPI proteins, colocalization has been confirmed by monitoring either the preferential partitioning of fluorescent GPI lipid probes with DIGs vs. bulk membranes [17,18] or the extensive fluorescence resonance energy transfer between GPI proteins [19]. Subsequent experimentation provided preliminary evidence that the packing order of the acyl chains, rather than their hydrophobicity, represents the predominant force for targeting lipid-modified proteins to DIGs [20]. On basis of these methods, partitioning of GPI proteins, such as Thy-1, into DIGs was demonstrated without the necessity of cross-linking.

Caveolae are small flask-shaped invaginations of the plasma membrane expressed in most terminally differentiated mammalian cell types [6,16]. They are devoid of clathrin coating and rich in cholesterol and (glyco)sphingolipids [21–24]. Caveolae presumably represent the 'invaginated type' of DIGs. Invagination presumably is driven by the abundant expression of caveolin 1–3 and flotillin, which fulfil a structural role (coating) and can be used as specific markers for caveolae [14,22]. Caveolae are particularly abundant in adipocytes and dramatically increase in number in parallel with their stage of differentiation from fibroblasts finally accounting for up to 20% of the cell surface area. Caveolae are involved in cellular transport processes (endocytosis, transcytosis, potocytosis, cholesterol transport, protein/lipid sorting), which may involve the recently characterized caveolae–caveosome–endoplasmic reticulum pathway, as well as in transmembrane signal transduction [22,24].

3. Microheterogeneity of DIGs

Recent data provide strong evidence for the existence of two distinct species of DIGs, DIGs of high cholesterol/caveolin content (hcDIGs) and DIGs of low cholesterol/caveolin content (lcDIGs). hcDIGs are characterized by lower buoyant density and higher cholesterol content as well as higher enrichment (vs. bulk plasma membranes) of caveolin and lipid-modified signalling proteins compared to lcDIGs which exhibit higher buoyant density and lower cholesterol/caveolin/signalling protein content. Both species coexist in 3T3-L1 fibroblasts and adipocytes as well as in rat adipocytes. Here, the major fraction of certain GPI proteins, i.e. GPI-anchored cAMP-binding ectoprotein-1 (Gce1) and 5′-nucleotidase (Nuc), as well as of the NRTK, $pp59^{Lyn}$, is located at hcDIGs [25]. In addition, the well-documented functional differences between Ras isoforms, H-Ras and K-Ras, may at least in part be due to their location at distinct plasma membrane microdomains [26,27]. H-Ras preferentially occurs in typical DIGs and K-Ras in different microdomains, which also resist solubilization by cold detergent but are insensitive toward cholesterol depletion. Both H- and K-Ras are prenylated, but H-Ras is additionally modified by palmitoylation, whereas K-Ras contains a basic polylysine sequence. This suggests that carboxy-terminal membrane anchors confer targeting specificity for distinct microdomains, i.e. palmitoylation plus prenylation for hcDIGs and polylysine plus prenylation for lcDIGs [20]. The results of a very recent study are compatible with this interpretation. Test proteins containing two different lipid (myristoyl and palmitoyl) chains became located in typical DIGs and associated with caveolin [28]. In contrast, test proteins harboring a prenyl (geranylgeranyl) tail were found clustered together in a microdomain species that does not depend on cholesterol and contains highly disordered lipids capable of accommodating the irregular structures of the prenyl tail [28]. Furthermore, in a previous morphometric analysis, the cholesterol content of DIGs was varied by the cholesterol-chelating agent, β-cyclodextrin. Microvillar vesicles from the enterocyte brush border membrane were immunogold-labeled and immunoisolated with antibodies directed toward galectin-4 which represents a marker specific for DIGs [29,30]. These experiments revealed that a 70−% reduction of microvillar cholesterol does not affect the localization of galectin-4 at DIGs. In fact, it has been demonstrated that microdomains can persist in the absence of cholesterol, whereas high cholesterol levels may even dissolve them [31]. Taken together, these findings strongly argue for the simultaneous presence of typical cholesterol-containing DIGs and DIGs of less pronounced or even missing cholesterol dependence. The detailed biochemical/morphological characterization of the latter, their identity with adipocyte lcDIGs and their presence in other differentiated cell types as well as the putative heterogeneity of caveolae remain to be determined.

4. Caveolin/caveolae signalling hypothesis

Cytoplasmic signalling proteins are targeted to DIGs/caveolae if they are (dually) acylated with long chain saturated fatty acids (see above). After arrival at DIGs/caveolae, they may directly interact with caveolin. A specific hydrophobic 20-aa domain within caveolin, the caveolin-scaffolding domain (CSD; aa 82–101 of caveolin-1; see legend to Fig. 2) has been identified which interacts with the corresponding caveolin-binding domain (CBD) of signalling proteins with high specificity [13]. The CBD consensus sequence has been delineated using a phage display library approach as ϕxϕxxxxϕxxϕ (ϕ = aromatic aa; x = any aa; see CBD of $pp59^{Lyn}$ in Fig. 1). Lisanti and coworkers demonstrated in vitro by using synthetic CSD peptide (CSDP) that the interaction between the CSD and the CBD of a signalling protein keeps this protein in the basal inactive state [13]. In response to certain stimuli, signalling proteins may be released from this complex with caveolin and thereby activated as stated in the so-called caveolin/caveolae signalling hypothesis [22]. Meanwhile convincing evidence has been obtained for operation of caveolin/caveolae signalling also in vivo in cells (see below) and animals. In this regard, a cell-permeable peptide containing the CSD, which interacts with the CBD of endothelial nitric oxide synthase, was found to potently inhibit acetylcholine-induced vasodilation and nitric oxide production in aortic rings and to suppress nitric oxide-mediated vascular compromise in mice treated with proinflammatory agents [32]. Furthermore, the findings that caveolin-1 can functionally interact with p42/44 mitogen-activated protein (MAP) kinases in vitro prompted investigations for such a regulation in the context of oncogenes under experimental conditions which lead to variations in caveolin-1 expression. In NIH3T3 cells upon oncogenic transformation with v-Abl and H-Ras (G12V) oncogenes, caveolin-1 was down-regulated, morphological caveolae were lost and simultaneously p42/44 MAP kinases were up-regulated [33,34]. Vice versa, upon heterologous expression of caveolin-1, caveolae were formed de novo and growth of the transformed cell lines

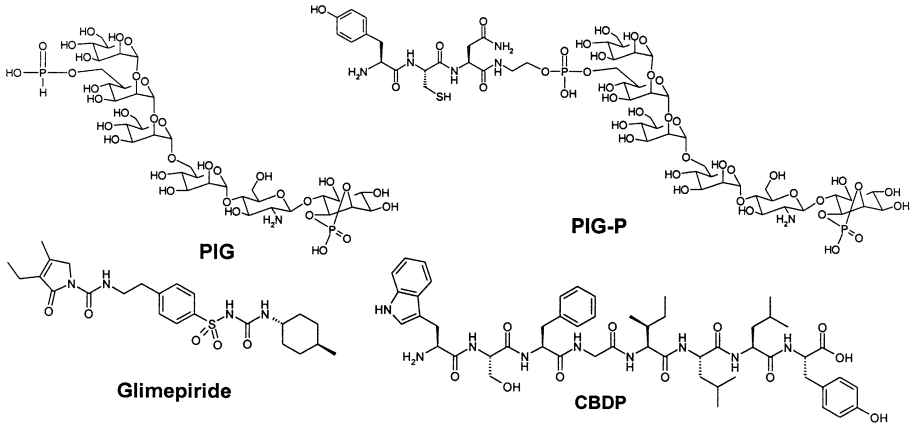

Fig. 1. Structures of stimuli triggering insulin-mimetic metabolic signalling and action in insulin target cells. PIG(-P) is derived from the lipolytically (by *Bacillus cereus* PI-specific phospholipase C) and proteolytically (by *Staphylococcus aureus* V8 protease) cleaved GPI protein, Gce1p, from *Saccharomyces cerevisiae*. In consequence, the termini consist of 2′,3′-cyclic phosphoinositol and tripeptidylethanolamidyl moieties; PIG is derived from PIG(-P) but lacks the tripeptidylethanolamidyl moiety and therefore represents the consensus core glycan head group of GPI proteins as conserved from yeast to man [65]; the sulfonylurea glimepiride is the ingredient of the antidiabetic drug amaryl (trade name); CBDP (WSFGILLY) is derived from the CBD of human pp59^{Lyn}.

became anchorage-dependent accompanied by inactivation of p42/44 MAP kinases [34].

The recently described generation of mice with a targeted disruption of the caveolin-1 locus provided first evidence for the consequences of caveolin deficiency in a mammalian system [35]. Mice lacking caveolin-1 were viable and fertile despite complete loss of morphological identifiable caveolae. This proved that caveolin-1 is absolutely required for the biogenesis of caveolae. Primary fibroblasts derived from caveolin-1 null mice proliferated faster than cells from their wild-type counterparts and had more active cell cycle profiles [35]. This negative regulation of the cell cycle and cell proliferation by caveolin-1 expression is in line with the caveolin/caveolae-signalling hypothesis since in caveolin-1 null mice certain cellular protooncogene products, such as c-Src, c-Myc, Neu tyrosine kinase [33–34,36], and components of the downstream signalling cascades, such as the Ras-p42/44 MAP kinase cascade, are no longer negatively controlled by association with caveolin-1. In fact, overexpression of caveolin-1 indicated that caveolin-1 is a potent inhibitor of the MAP kinase cascade [37]. Furthermore, antisense-mediated down-regulation of caveolin-1 in NIH3T3 cells led to hyperactivation and transformation of these cells [38]. Finally, ablation of caveolin expression in *Caenorhabditis elegans* via RNAi caused hyperactivation of the meiotic cell cycle, resembling the phenotype of uncontrolled Ras signalling [39].

5. Insulin signalling via DIGs/caveolae

Insulin receptor signal transduction has been demonstrated to depend on functional caveolae [40,41]. Lowering of the cholesterol content in adipocyte plasma membranes by cho-

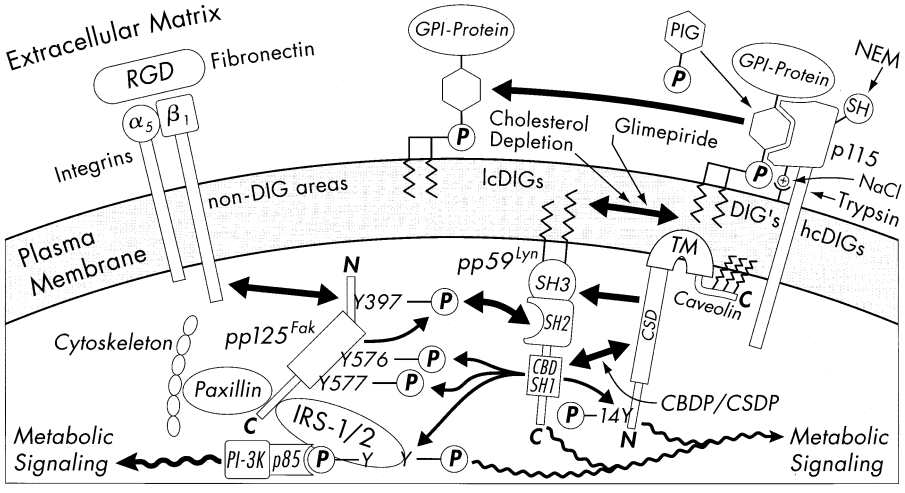

Fig. 2. Hypothetical mechanism for insulin-mimetic signalling via induction of redistribution of signalling proteins between plasma membrane microdomains (see text for details) for which the term 'GPI/PIG signalling' is proposed. Thick uni-directional arrows, redistribution of signalling proteins (GPI proteins and pp59^{Lyn}) from hcDIGs to lcDIGs; thick bi-directional arrows, protein/protein-, protein/lipid- and lipid/lipid-interactions; medium-sized arrows, tyrosine (auto)phosphorylations; thin arrows, molecular targets of insulin-mimetic stimuli (PIG-P, glimepiride, CBDP) and of inhibitors for redistribution/activation of signalling proteins (sequential trypsin and NaCl treatment, NEM treatment, cholesterol depletion, CSDP = DGIWKASFTTFTVTKYWFYR); wave-lined arrows, downstream signalling to metabolic effector systems.

lesterol extraction with β-cyclodextrin or enzymatic cholesterol oxidation resulted in loss of the structural integrity of caveolae without cell rupture. This was paralleled by progressive inhibition of tyrosine phosphorylation of insulin receptor substrate protein (IRS) 1 and activation of glucose transport in response to insulin although autophosphorylation of the insulin receptor was not impaired [40,41]. Thus, caveolae/DIGs seem to act as the structural platform required for efficient coupling of the insulin receptor tyrosine kinase to IRS in insulin target cells. Recently, a distinct pathway for regulated translocation of signalling proteins from bulk plasma membranes to DIGs has been elucidated in cultured adipocytes [42]. The insulin-dependent tyrosine phosphorylation of Cbl was demonstrated to recruit the APS/CAP/Cbl/CrkII/C3G multiprotein complex to DIGs where the C3G component regulates a small GTP-binding protein, TC10, that exclusively occurs at DIGs. The interaction of the complex with DIGs and, in consequence, the exposure of Cbl to the insulin receptor (a fraction of which resides in caveolae) relied on specific binding of the amino-terminal domain of CAP to the DIGs-resident protein, flotillin [43]. Deletion analysis of the CAP gene identified a 115-aa sorbin homology domain responsible for flotillin binding [44]. Overexpression of a CAP mutant lacking this domain blocked translocation of the signalling complex to DIGs and simultaneously abrogated insulin stimulation of glucose transport. These data suggest that in adipocytes at least two types of protein–protein interaction may determine the targeting of cytoplasmic signalling proteins to DIGs and their partitioning between DIGs and plasma membranes, (i) interactions between flotillin and the sorbin homology domain and (ii) interactions between caveolin and the CBD. However, the relative importance of the two pathways for both differential recruitment of signalling proteins to DIGs and to metabolic insulin signalling in other insulin-responsive cell types has to be confirmed and the molecular mechanisms of the putative cross-talks to each other as well as to the insulin signalling cascade remain to be studied.

6. Insulin-mimetic signalling via DIGs

Previous studies have identified three completely different exogenous stimuli (Fig. 1) which induce tyrosine phosphorylation of IRS and glucose transport activation in insulin-sensitive target cells. They dispense with the activation of the insulin receptor, but rather act by triggering redistribution of signalling components between hcDIGs and lcDIGs. (i) Synthetic phosphoinositolglycan(-peptides)(PIG(-P)) and PIG(-P) isolated from natural sources provoke insulin-mimetic metabolic actions in insulin-responsive and insulin-resistant adipose and muscle cells in vitro [45–47]. PIG(-P) are derived from the polar core glycan head group of GPI proteins. They consist of 2′,3′-cyclic phospho-*myo*-inositol coupled to five sugar residues in typical glycosidic linkages (PIG portion) and, optionally, contain an additional terminal ethanolamine-linked tripeptide residue (P portion). In adipocytes isolated from insulin-resistant Zucker fatty rats, PIG(-P) displayed higher potency in stimulating non-oxidative glucose metabolism and cell surface expression of the insulin-responsive glucose transporter, GLUT4, than insulin [48]. (ii) The hypoglycemic sulfonylurea drug, glimepiride, which exerts a moderate insulin-independent blood glucose-decreasing activity in type II diabetic animals and patients, has been demonstrated to induce glucose transport and GLUT4 translocation in isolated insulin-resistant rat adipocytes. In parallel, tyrosine phosphorylation of IRS-1/2 and insulin-mimetic regulation of the typical insulin signalling components downstream of IRS, such as PI3K, PKB and GSK-3β, were observed [49]. (iii) Introduction of synthetic CBD peptide (CBDP; derived from $pp59^{Lyn}$) into isolated rat adipocytes by electroporation was shown to stimulate tyrosine phosphorylation of IRS-1, PI3K activity and glucose transport [50].

7. Dynamics of microdomains and GPI/PIG signalling as target for insulin-mimetic stimuli

In an effort to identify the primary target of PIG(-P) in insulin target cells, e.g. a putative receptor, we found that sequential incubation of adipocytes with trypsin and NaCl or incubation with *N*-ethylmaleimide (NEM) drastically reduced insulin-mimetic signalling and action by PIG(-P)[51]. Reconstitution experiments in which trypsin/NaCl-treated cells had been reconstituted with the trypsin/NaCl extract identified a 115-kDa polypeptide as candidate for the trypsin/NaCl/NEM-sensitive component operating at or upstream of IRS tyrosine phosphorylation. Subsequent studies demonstrated specific and saturable binding of radiolabeled PIG-P, as well as of a radiolabeled and a lipolytically cleaved yeast GPI protein (harboring the terminal PIG[-P] moiety) to hcDIGs [52]. Binding to hcDIGs was abolished after inactivation of trypsin/NaCl/NEM-sensitive p115, but considerably increased after pretreatment of intact adipocytes with (G)PI-specific phospholipase C. These data suggest that in rat adipocytes insulin-mimetic PIG(-P) are recognized by trypsin/NaCl/NEM-sensitive p115 located at hcDIGs. p115 might act as physiological receptor for endogenous ligands, such as GPI proteins/lipids. A strict correlation has been found between specific binding of synthetic PIG(-P) derivatives to the PIG(-P) receptor and their insulin-mimetic signalling/metabolic activity [53].

In an effort to identify the primary target of glimepiride in insulin target cells, for instance a putative receptor, we found that in intact rat adipocytes or isolated DIGs authentic radiolabeled glimepiride does not interact specifically with a cell surface protein but is photo-cross-linked to two distinct GPI lipid species enriched at hcDIGs in non-saturable fashion ([54]; N. Hanekop, G. Müller, unpublished data). Apparently, hydrophilic PIG(-P) and lipophilic glimepiride interact with hcDIGs of the adipocyte plasma membrane in fundamentally different fashion. Presumably, PIG(-P) specifically bind to the PIG(-P) receptor, whereas glimepiride spontaneously intercalates between GPI lipids.

The presence of NRTKs of the Src class in DIGs/caveolae in insulin target cells and their regulation by caveolin (see above) raised the possibility that $pp59^{Lyn}$ mediates tyrosine phosphorylation induced by PIG(-P), glimepiride or CBDP. In fact, introduction of neutralizing anti-$pp59^{Lyn}$ antibody or excess of synthetic wildtype CSDP into isolated rat adipocytes by electroporation blocked IRS-1 and $pp59^{Lyn}$ tyrosine phosphorylation and glucose transport activation in response to PIG(-P)[50]. In agreement with the caveolin/caveolae signalling hypothesis (see above), this may be due to direct binding of the inhibitory CSDP to the CBD of $pp59^{Lyn}$ and subsequent blocking of its activation. In basal adipocytes, caveolin and $pp59^{Lyn}$ can be coimmunoprecipitated as one complex

from solubilized DIGs demonstrating their direct interaction (rather than mere colocalization within DIGs) which keeps pp59^{Lyn} in the inactive state [55]. Introduction of CBDP into these adipocytes led to displacement of pp59^{Lyn} (and also pp125^{Fak}) from caveolin concomitant with increased tyrosine phosphorylation of IRS-1, which is presumably based on relief of pp59^{Lyn} from inhibition by caveolin. Strikingly, PIG(-P) and glimepiride also induce release of pp59^{Lyn} and pp125^{Fak} from caveolin at hcDIGs and promote the translocation of the NRTKs to lcDIGs. This is accompanied by redistribution of the GPI proteins, Gce1 and Nuc, from hcDIGs to lcDIGs of the adipocyte plasma membrane [56]. Thus, activation of pp59^{Lyn} seems to be based both on its dissociation from caveolin left at hcDIGs and proper incorporation at lcDIGs. Disruption of DIGs by cholesterol depletion of the adipocyte plasma membrane led to almost complete blockade of PIG(-P)-, glimepiride- and CBDP-induced redistribution of Gce1, pp125^{Fak} and pp59^{Lyn} from hcDIGs to lcDIGs, tyrosine phosphorylation of pp59^{Lyn} and IRS-1 as well as activation of glucose transport. This strongly argues for a causal relationship between these processes [25]. Thus, signalling to IRS by insulin, PIG(-P), CBDP and glimepiride apparently shares the involvement of microdomains (hcDIGs, caveolae) and the redistribution of signalling proteins between the bulk plasma membrane and DIGs (insulin) or within DIGs (PIG-[P], CBDP, glimepiride). However, the molecular mechanisms which underlie signalling to IRS by these stimuli seem to differ on basis of the differential engagement of pp59^{Lyn} (in PIG-P, CBDP, glimepiride, but not insulin signalling) and of trypsin/NaCl/NEM-sensitive p115 (in PIG-P signalling, only)[25].

8. Hypothetical mechanism for partitioning of GPI proteins between DIGs and its cross-talk to insulin-mimetic signalling

Targeting of proteins to DIGs is currently assumed to be based solely on their lipid modification ([dual] acylation or glypiation, see above) and to be independent of direct interaction with microdomain-resident proteins, such as caveolin and flotillin [17,57,58]. However, the recent findings on the molecular mode of PIG(-P) action suggest a role of the PIG(-P) receptor in regulating the partitioning of GPI proteins between distinct plasma membrane microdomains: in the absence of a PIG(-P) receptor, GPI proteins spontaneously partition between bulk plasma membranes, lcDIGs and hcDIGs with some preference for lcDIGs (due to their lipid modification). This equilibrium is dramatically shifted to hcDIGs upon specific binding of the PIG(-P) epitope of the GPI anchor to the PIG(-P) receptor (Fig. 2). In conclusion, the saturated acyl chains of the GPI anchor seem to be necessary and sufficient for accumulation in lcDIGs compared to bulk membranes. The PIG(-P) moiety of the GPI anchor, on the other hand, may favor lateral movement of GPI proteins from lcDIGs to hcDIGs.

The observed redistribution of GPI proteins within DIGs of the adipocyte plasma membrane in response to PIG(-P), glimepiride and CBDP can be incorporated into the following working model for insulin receptor-independent, microdomain-dependent insulin-mimetic signalling in insulin target cells (Fig. 2). (i) PIG(-P) bind as agonists/antagonists to the PIG(-P) receptor. This protein presumably is identical with trypsin/NaCl/NEM-sensitive p115 being anchored at the cell surface via a trypsin-sensitive transmembrane domain and salt bridges. Upon binding, it displaces those GPI proteins from the PIG(-P) receptor which are already bound there. (ii) Glimepiride inserts into DIGs via hydrophobic interactions with GPI lipids thereby altering the structural organization of hcDIGs. Both events induce the redistribution of GPI proteins and NRTKs from hcDIGs to lcDIGs accompanied by dissociation of the NRTKs from caveolin. (iii) CBDP directly causes dissociation of pp59^{Lyn} from caveolin (whereas CSDP binds to and inhibits pp59^{Lyn}). PIG(-P)-, glimepiride- or CBDP-induced release of pp59^{Lyn} from caveolin/hcDIGs leads to its activation and to hierarchical phosphorylation of pp125^{Fak} at Y576/577 (by pp59^{Lyn}) and subsequent autophosphorylation of pp125^{Fak} at Y397. After complex formation with cytoskeletal components (paxillin) and contact to the extracellular matrix (via integrins and fibronectin), pp125^{Fak} operates as platform molecule presenting IRS-1/2 for tyrosine phosphorylation by activated pp59^{Lyn} at sites recognized by the p85 subunit of PI3K and possibly at additional sites. Furthermore, pp59^{Lyn} has been shown to phosphorylate caveolin (at Y14) and may recognize additional substrates, such as Cbl, from which IRS/PI3K-independent pathways are initiated. Ultimately, activation of IRS/PI3K-dependent and -independent pathways trigger signalling to the full panel of metabolic insulin effector systems, such as the GLUT4 translocation and antilipolysis machineries.

9. Communication across the membrane leaflets of microdomains

According to the present data, dissociation of the GPI proteins, Gce1 and Nuc, from the PIG(-P) receptor seems to be obligatorily linked to dissociation of pp59^{Lyn} from its binding protein, caveolin, enabling parallel movement of GPI proteins and NRTKs from hcDIGs to lcDIGs. The underlying molecular mechanism remains enigmatic since the acyl groups of GPI proteins and NRTKs are restricted to the exoplasmic and cytoplasmic leaflets, respectively, of the plasma membrane lipid bilayer. It is conceivable that direct contacts between the fatty acid moieties of (redistributing) GPI proteins and those of caveolin-associated glycerophospholipids of the cytoplasmic leaflets of DIGs generate a signal for caveolin which causes down-regulation of its binding affinity for NRTKs. In line with this mechanism, protein–protein interactions between GPI proteins and caveolin or GPI proteins and pp59^{Lyn} have not been described so far (see also [56]). Alternatively, the association of caveolin-1 with cholesterol, which is dependent on its palmitoylation, may link the cytoplasmic and exoplasmic leaflets of the bilayer together. Cholesterol is present in both leaflets and can potentially form dimers that span the membrane, acting as a bridge [59]. Thus, caveolin-1 complexed with cholesterol may function as the postulated linker mediating transmembrane signalling from GPI proteins to NRTKs which is restricted to DIGs. This function may also be fulfilled by other proteolipids, i.e. microdomain transmembrane adaptor proteins, that contain palmitoyl groups, such as LAT and PAG [60]. Finally, it cannot be excluded that redistribution of GPI proteins from hcDIGs to lcDIGs triggers in pp59^{Lyn} the substitution of palmitate for long chain unsaturated fatty acids impeding its localization at hcDIGs. A less hydrophobic or less ordered lipid anchor would be predicted to force acylated proteins

into less tightly packed microdomains, such as lcDIGs, or the bulk plasma membrane. This has been demonstrated previously for the *S*-acylated NRTK, Fyn, in T cells [61]. A prerequisite for operation of this mechanism would be a high turnover rate of palmitate on pp59^{Lyn}. In fact, this may be true according to the observation that in Jurkat cells [^{3}H]palmitate on the NRTK, Lck, has a (relatively short) half-life of 1–2 h [62]. These findings together with the reports that introduction of an unsaturated acyl chain into the G protein, $G_{\alpha I}$, [63] and of the heteroatom-substituted palmitic acid analogue, 13-oxypalmitic acid, into Lck impairs their association with hcDIGs [62] suggest that the nature of the fatty acid, by which a signalling protein is modified, regulates their partitioning between the distinct microdomains, hcDIGs and lcDIGs, and the bulk plasma membrane and determines their final localization. However, this mechanism leaves unanswered the question how redistribution of GPI proteins at the outer leaflet signals activation to the relevant protein: *S*-acyl transferase acting at the inner leaflet. In this regard, it seems more plausible that the loss of long chain saturated fatty acids from the outer leaflet caused by the redistribution of GPI proteins from hcDIGs to lcDIGs simultaneously alters the fatty acid composition of the microdomains at the inner leaflet, too. This 'adjustment to a common fatty acid milieu' across the leaflets of a microdomain may facilitate or be supported by the redistribution of pp59^{Lyn} from hcDIGs to lcDIGs. A similar impact of the fatty acid composition on membrane targeting has been suggested for the displacement of Src family kinases from microdomains [64].

10. Microdomains and GPI/PIG signalling as targets for pharmacological intervention

The pathogenesis of type II diabetes and metabolic syndrome is characterized by significant impairment of insulin signalling downstream of the insulin receptor tyrosine kinase. In particular, IRS tyrosine phosphorylation and the resulting activation of PI3K-dependent and -independent pathways in muscle and adipose cells are severely hampered. Consequently, small drug molecules provoking insulin/insulin receptor-independent tyrosine phosphorylation of IRS via effecting redistribution of GPI proteins and NRTKs between hc/lcDIGs in insulin target cells may bypass insulin resistance and thereby lower blood glucose in type II diabetic patients. Such drugs may be discovered by one of the following strategies, provided the inherent more or less pronounced bottlenecks can be overcome: (i) blockade of the interaction between the CBD of pp59^{Lyn} and the CSD has to address the complex and ill-defined area of protein–protein interactions, (ii) intercalation into DIGs, as exemplified by glimepiride, has to ensure specificity and involve a stoichiometric rather than catalytic mechanism, (iii) down-regulation of the PIG(-P) receptor represents a so-called 'black box' (transcription-based) rather than molecularly defined target and, presumably most attractive, (iv) agonism/antagonism of the PIG(-P) receptor offers the advantage of guaranteeing high specificity and potency but requires the analysis of large surface areas covered by protein–glycan interactions. In any case, future antidiabetic drugs identified thereby may demonstrate drug-induced redistribution of signalling proteins between plasma membrane microdomains as a novel and attractive principle for signal transduction therapy.

Acknowledgements: I thank Drs. Wendelin Frick, Stefan Petry and Werner Kramer for continuous outstanding intellectual and experimental support, Christian Jung, Susanne Wied, Stefan Welte and Holger Jordan (all Aventis Pharma Germany) for excellent technical assistence and Dr. Wolfhard Bandlow (University Munich, Germany) for very fruitful discussions during progression of the experimental work presented in this review and for helpful advice during preparation of the manuscript.

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